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Rhodamine-123 Selectively Reduces Clonal Growth of Carcinoma Cells in vitro

Abstract. *Rhodamine-123, a cationic laser dye, markedly reduced the clonal growth of carcinoma cells but had little effect on nontumorigenic epithelial cells in vitro. This selective inhibitory effect of Rhodamine-123 on some carcinomas is unusual since known anticancer drugs, such as arabinosyl cytosine and methotrexate, have not been shown to exhibit such selectivity in vitro.*

The fluorescent dye Rhodamine-123 (Rh-123) selectively accumulates in the mitochondria of living cells (*1*). The specific accumulation of this dye appears to depend on its cationic and permeant properties and on the high electric potential (inside negative) across the mitochondrial membrane (*2, 3*). Although all living cells we have examined thus far accumulate Rh-123 in their mitochondria, cell types differ in their ability to retain Rh-123 in dye-free medium (*3, 4*). We found that most carcinoma cells retain Rh-123 for 2 to 5 days when they are incubated in dye-free medium, whereas nontumorigenic epithelial cells and tumorigenic or nontumorigenic cells of fibroblastic, neural, or hematopoietic origin release the dye within 1 to 16 hours (*4*). It may be possible to exploit the difference in Rh-123 retention between carcinoma cells and other cell types for cancer chemotherapy (*5*). In the study described herein, we compared the effects of Rh-123 treatment on the clonal growth of carcinoma cells and nontumorigenic epithelial cells in vitro.

For these experiments we used MB 49 cells, a mouse bladder epithelial line transformed by 7,12-dimethylbenz[*a*]anthracene (DMBA) (*6*). These cells are highly tumorigenic and retain a significant amount of Rh-123 in their mitochondria for 4 days when they are incubated in dye-free medium. After 2 weeks, the numbers of colonies were counted and the results were expressed as percentages of control, with control plates normalized to 100 percent CFU. The standard error for triplicate samples was 3 to 5 percent. The MB 49 cells (a mouse bladder epithelial cell line transformed with DMBA) and EJ cells (human bladder carcinoma cell line) were plated and treated with different concentrations of Rh-123 for various times before they were washed and reincubated in fresh medium. The CFU (percentage of control) was determined as described above. The standard error for duplicate samples was 5 percent.

cultures of normal bladder epithelial cells lose Rh-123 fluorescence within 2 hours. To determine whether this difference in retention results in greater inhibition of clonal growth of MB 49 cells than of normal mouse bladder epithelial cells, we treated these cells grown in vitro with Rh-123 and assayed their colony-forming ability. Exposure to Rh-123 (10 μg/ml) for 24 hours had a minimal effect on the colony-forming units (CFU) of normal mouse bladder epithelial cells (92 percent of control) (Fig. 1A), but markedly reduced the CFU of MB 49 cells (4 percent of control). The effect of Rh-123 on MB 49 cells depended on the concentration and duration of exposure. Even 6 hours of exposure to 10 μg of Rh-123 per milliliter reduced the CFU to 45 percent of control, whereas such treatment had no significant effect on the CFU of normal bladder epithelial cells.

We then compared the reductions in CFU of EJ cells (a human bladder carcinoma line), MB 49 cells, and normal mouse bladder epithelial cells that had been exposed for 24 hours or continuously to different concentrations of Rh-123 during the 2-week period of clonal cell growth (Fig. 1, B and C). Continuous exposure of normal mouse bladder epithelial cells to Rh-123 (10 μg/ml) had only a small effect on CFU. However, both EJ and MB 49 cells were susceptible to the inhibitory effects of Rh-123. Colony formation in these cells was reduced to 50 percent of control after 24 hours of exposure to 2 to 5 μg of Rh-123

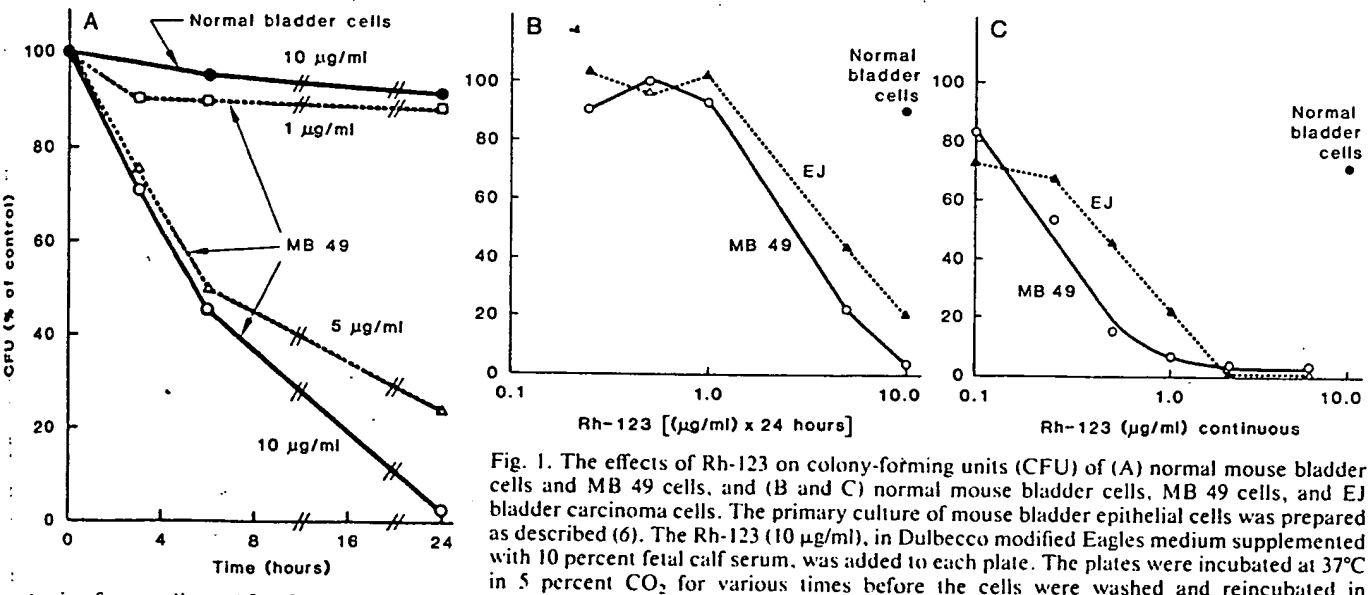

Fig. 1. The effects of Rh-123 on colony-forming units (CFU) of (A) normal mouse bladder cells and MB 49 cells, and (B and C) normal mouse bladder cells, MB 49 cells, and EJ bladder carcinoma cells. The primary culture of mouse bladder epithelial cells was prepared as described (*6*). The Rh-123 (10 μg/ml), in Dulbecco modified Eagles medium supplemented with 10 percent fetal calf serum, was added to each plate. The plates were incubated at 37°C in 5 percent CO_2 for various times before the cells were washed and reincubated in rhodamine-free medium. After 2 weeks, the numbers of colonies were counted and the results were expressed as percentages of control, with control plates normalized to 100 percent CFU. The standard error for triplicate samples was 3 to 5 percent. The MB 49 cells (a mouse bladder epithelial cell line transformed with DMBA) and EJ cells (human bladder carcinoma cell line) were plated and treated with different concentrations of Rh-123 for various times before they were washed and reincubated in fresh medium. The CFU (percentage of control) was determined as described above. The standard error for duplicate samples was 5 percent.

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per milliliter (Fig. 1B) and by continuous exposure to 0.2 to 0.5 μg of Rh-123 per milliliter (Fig. 1C).

We also compared the effects of Rh-123 on the CFU of other carcinoma and nontumorigenic cell lines, all of which have been confirmed to be of epithelial origin by studies of immunofluorescence with keratin antibody. The carcinoma cells retained Rh-123 longer than nontumorigenic epithelial cells (*4*). BSC 1, a nontumorigenic line of monkey kidney epithelial cells (Fig. 2A) and CCL 34, a nontumorigenic dog kidney epithelial line (Fig. 2B) were relatively insensitive to Rh-123. In contrast, CCL 51, a mouse breast carcinoma line (Fig. 2A) and HUT 23, a human lung adenocarcinoma line (Fig. 2B), were very sensitive to the inhibitory effects of Rh-123, with colony formation being reduced to 50 percent of control by continuous exposure to 0.2 to 0.5 μg of Rh-123 per milliliter.

The clonal growth of MCF-7 cells (human breast carcinoma line) and Ehrlich ascites cells (mouse carcinoma line) was also reduced to 50 percent of control after continuous exposure to Rh-123 (0.5 μg/ml), whereas the clonal growth of PtK1 (a nontumorigenic marsupial kidney line) and CRL 1521 (a normal human skin fibroblast line) was greater than 80 percent under similar conditions. However, the clonal growth of all the cell lines we tested was not reduced by [illegible] minutes of exposure to 10 μg of Rh-123 per milliliter. Thus, the conditions required for specific staining of mitochondria (*1*, *2*), for measurements of Rh-123 retention (*3*, *4*), and for cell viability assays (*3*, *7*) were not inhibitory to the cells.

Unlike Rh-123, arabinosyl cytosine (Ara-C) and methotrexate (cell cycle–specific anticancer drugs) were not selectively inhibitory for carcinoma cells in vitro (Fig. 2, C and D). Since BSC 1, CCL 34, CCL 51, and HUT 23 had similar doubling times, it appeared that Ara-C and methotrexate inhibited the clonal growth of cycling tumorigenic and nontumorigenic epithelial cells. These results also suggest that the selective inhibition of CFU by Rh-123 was not due to differences in cell-cycle kinetics between carcinoma and nontumorigenic epithelial cells.

The ability of carcinoma lines to retain Rh-123 may have been important in their sensitivity to Rh-123. However, this prolonged dye retention was unlikely to have been the sole mechanism for the selective inhibitory effects since continuous treatment with Rh-123 also resulted in a much greater reduction of CFU in carcinoma cells than in nontumorigenic epithelial cells. Some possible explanations for these observations are (i) that certain cellular components or organelles (possibly mitochondria) of carcinomas are very sensitive to the inhibitory effects of Rh-123; (ii) that nontumorigenic cells have an active mechanism for excluding Rh-123 from the vulnerable cellular compartment in the face of continued presence of Rh-123 in the culture medium; or (iii) that nontumorigenic cells are capable of inactivating Rh-123. Nonetheless, mitochondria are the likely targets for the inhibitory action of Rh-123 since they selectively accumulate the dye (*1–3*). Rhodamine-123 may disrupt some mitochondrial functions, such as the translocation of adenosine diphosphate (*8*), proton ejection (*9*), or electron transport (*10*). In previous studies the cytostatic effects of Rh-123 on the L 1210 leukemia line (*11*) and of rhodamine 6G on the mink fibroblast line CCL 64 (*12*) were described.

Tumors of the lung, breast, and colon are still the major causes of deaths due to cancer in the United States (*13*), and new and more selective drugs are needed to combat these tumors. The system we have described in this report would be useful for screening the anticarcinoma activity of additional rhodamine analogs before they are tested in animals. It would be of interest to determine whether some carcinomas, particularly those that do not retain Rh-123 (*4*), are resistant to the inhibitory effects of Rh-123. Whether Rh-123 can prolong the survival of mice implanted with carcinomas also remains to be investigated.

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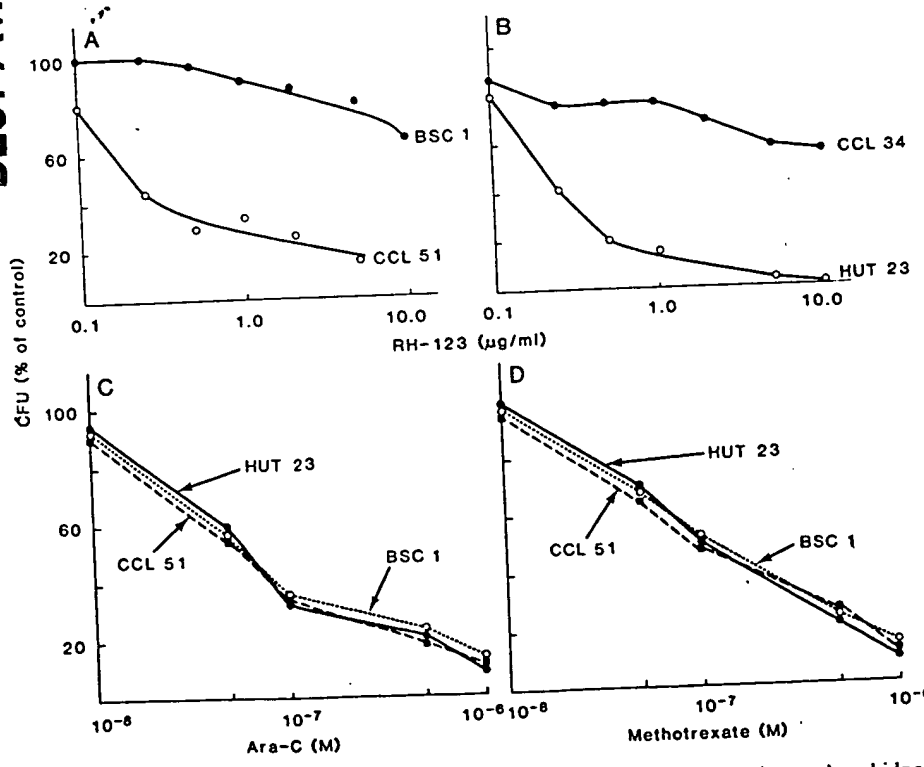

Fig. 2. The effect of Rh-123 on CFU of (A) BSC 1 cells, a nontumorigenic monkey kidney epithelial line, and CCL 51 cells, a mouse breast carcinoma line, and (B) CCL 34 cells, a nontumorigenic dog kidney epithelial line, and HUT 23 cells, a human lung adenocarcinoma line. The effect of (C) Ara-C and (D) methotrexate on CFU of BSC 1, CCL 51, and HUT 23 cell lines.

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